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Research Article

Comparative analysis of environmental arsenic and other heavy metals in 2 Buruli Ulcer endemic districts in the Ashanti Region, Ghana

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Abstract
Arsenic occurs naturally and ranks as 20th most abundant trace element in the earth's crust. It has been linked to Buruli Ulcer Infections in recent times. Arsenic and other heavy metals in the Asanti Akim North District were assessed and compared with concentrations recorded in Amansie West District. Analysis of results showed that, arsenic concentration in sampled streams within the Asanti Akim North District was 0.1545mgL^{-1}. This exceeded the WHO and GEPA recommendation for drinking water. Analysis of the results however, did not show any significant difference in terms of arsenic recorded from streams in Amansie West and Asanti Akim. Arsenic levels in the Amansie West were greater (0.1772 $\pm$ 0.07323mgL^{-1}) than levels recorded from Ashanti Akim North District (0.1545 $\pm$ 0.0503mgL^{-1}). Cadmium levels were analysed in water and soil based on the district designation and the results showed that, Amansie West District had levels in streams (0.0568 $\pm$ 0.0045mgL^{-1}) that was less than that of Asanti Akim North (0.0600 $\pm$ 0.0074 mgL^{-1}) (p= 0.7185). Result from this pilot study has shown that the Asanti Akim North District is also polluted with arsenic just as the Amansie West, affirming a possible link to BU infection.

Keywords
Asanti Akim North — Amansie West — cadmium — lead — infections

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Content

Background

Arsenic occurs naturally and ranks as 20th most abundant trace element in the earth's crust [1] and is widely distributed in the environment. Its association with some non-weathering-resistant mineral deposits (e.g., sulphide minerals) has contributed to its release in large amounts into the environment [2]. Arsenic exists mainly in three valency states (i.e., $^{-3}$,$^{+3}$, $^{+5}$). The tri-valent arsenic (As^{3+}) and the penta-valent arsenic (As5+) are widely present in natural waters and are soluble over a wide range of pH and Eh conditions [3]. Organic arsenical compounds exist but these are generally low but not irrelevant toxicological significance [4]. Under anaerobic conditions, arsenite can be reduced to arsine by microorganisms in soil [5]. Arsenic species may be methylated as monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO) by microorganisms, humans and animals [6]. Arsenic is used in hardening of alloys and in production of pigments, semiconductors, rodenticides, fungicides etc [7]. Because of its usefulness and exploitation, contamination of this heavy metal is now rampant in the environment.

Other heavy metals that found in the environment include cadmium and lead among many others. Cadmium is an extremely toxic metal commonly found in industrial workplaces

[8]. Due to its low acceptable exposure levels, overexposures may even be possible in situations where trace quantities of cadmium are detected. Cadmium is now being employed in electroplating, though the actual nature of the operation is not known lead to overexposures generally. Lead on the other hand at high concentrations can potentially be toxic to humans and animals. In view of the above, a study on the level of pollution of this heavy metal is, particularly in communities where lead contaminated water is used for agricultural purposes [9]. *Mycobacterium ulcerans* infection otherwise known as Buruli Ulcer is an ulcerative disease of the skin, found in rural areas located near wetlands (ponds, swamps, marshes, impoundments, backwaters) [10]. The causative organism is from the family of bacteria which causes tuberculosis and leprosy but this mycobacterium disease has received less attention than these diseases [11]. Infection leads to extensive destruction of skin and soft tissue with the formation of large ulcers usually on the legs or arms [10]. Patients who are not treated early often suffer long-term functional disability such as restriction of joint movement as well as the obvious cosmetic problem [12]. Arsenic has been linked to Buruli Ulcer infections in recent times [13] [14] where arsenic was monitored over a period of 24 months in Buruli Ulcer endemic community of the Amansie West District, Ghana. In confirming the involvement of arsenic exposure to BU infections, experimental mice were given water containing arsenic at concentrations synonymous to Buruli Ulcer endemic communities of the Amansie West District. Results of the study revealed that, arsenic in drinking water at levels ranging between 0.8 to 4.8 mg/L made mice susceptible to Buruli Ulcer infection. It is important to note that in spite of the plethora of studies carried out by numerous researchers globally in Buruli Ulcer infections including the World Health Organization (WHO) in recent times, the pathogenesis of this mysterious skin disease remains unclear. There have been numerous accounts by patients in the Amansie West (Unpublished data) who claim to have suffered BU in months after drinking water from streams in their locality. To draw a stronger conclusion for the involvement of arsenic in BU pathogenesis, a study to monitor arsenic in other BU districts outside the Amansie West District is urgently warranted. In this present study, arsenic and other heavy metals were monitored for 6 BU endemic communities in the Asanti Akim North District. This would be helpful in comparing BU infections to arsenic exposure in the environment.

1. Materials and Methods

1.1 *Asanti Akim North District*

Buruli Ulcer Disease has been prevalent in the Asante Akim North District of Ghana since 1971, several years before the BU became endemic in the country and the West African Sub Region in the early 1990_s. Initially the disease was limited to villages in the Afram Plains sector of the district but it gradually spread to other towns in the district [15]. The Asante Akim North District is one of the Districts in the Ashanti Region of Ghana. The district is located in the eastern part of the Ashanti Region and lies between latitude 60 30' North, 70 30' North and longitude 00 15' West, 10 20' West. It covers a land area of 1,160 $_{km}{}^3$ with an estimated population of 169,976 for the year 2006 (Projection from 2000 Population Census).

1.2 Study Areas and Population:

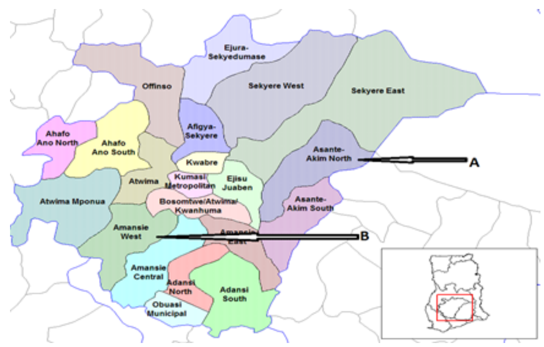

Figure 1. A Map of Ashanti Region of Ghana showing 2 districts (arrowed A & B) (Left) with Amansie West (B) and Asanti Akim North (B) as well as the map of Ghana (Right) insect.

Source: `www.ij-healthgeographics.com`

Study Communities Six (6) communities were systematically selected based on reports of BU in the community in the last three years. Endemic communities chosen were Dukusen, Srebuoso and Ananekrom whiles the non-endemic communities' were Pataban, Supinisu and Agogo.

(a)Water sampling After shaking vigorous stream where sampling is to be done, water was taken into pre labeled plastic containers and tightly covered. All physicochemical parameters were measured in situ with methods described by Gyasi *et al.,* with slight modification [14]. Stream water meant for analysis were acidified with few drops of concentrated sulphuric acid for every 500_{ml} sample on site. They were capped and stored in a cool ice chest and transported for analysis in the laboratory.

(b)Soil sampling Composite sampling was employed for soil collection from designated sites with methods described by *Gyasi et al.,* with slight modification. They were then correctly labeled, stored in plastic bags, kept in a cool ice chest and sent to the laboratory for analysis [14].

1.3 Detection of Arsenic, Cadmium and Lead

The measurements of arsenic and other heavy metals were performed using a PerkinElmer® PinAAcle^{TM} 900T atomic absorption Spectro-Photometer(AAS) (Shelton, CT, USA). This instrument is equipped with the intuitive WinLab32^{TM} for AA software for optimal performance. The heavy metal detection

equipment was coupled to a FIAS 400 flow injection analysis system that incorporates two peristaltic pumps, a 5-port flow injection valve and a regulated gas supply. Default parameters found in the software were used for all three elements: As, Cd, and Pb. Using a FIAS-AAS system, a sample loop on the flow injection valve was filled with the acidified sample, blank, or standard. The valve was then automatically switched to the inject position and the sample was mixed with a pumped stream of reductant, sodium borohydride for hydrides or stannous chloride.

At the point of reaction with the reductant, arsenic, cadmium and elemental lead were produced, along with hydrogen from the sodium borohydride, resulting in a two-phase mixture. A flow of argon was added to this mixture and the vapors were carried through a gas/liquid separator. This allowed the gaseous phase which contained the analyte vapor to enter the quartz cell on the AAS for analysis while the remaining liquids were pumped to a waste container. Values detected for arsenic, cadmium and lead were recorded accordingly.

1.4 Data Analysis

With the help of GraphPad statistical soft ware, means of As, Cd and Pb from both endemic and non endemic communities levels within the Asanti Akim North District were compared using chi-square non parametric analysis at 99% confidence intervals. This was also done for levels of these heavy metals detected from the two Buruli Ulcer endemic communities in the Ashanti region of Ghana.

2. Results

Analysis of results from the study showed that, mean arsenic concentration in water collected from streams within the Asanti Akim North District was 0.1545mgL with a standard deviation of 0.1743. This figure clearly exceeded the World Health Organization (WHO) and Ghana environmental Protection Agency (GEPA) recommendation for arsenic in drinking water. The study also showed that, mean concentration of arsenic in sediments from streams was also 176.4uSL^{-1}with a mean Total Dissolved Solid (TDS) of 92.46mgL^{-1}. These were however within the limits recommended by WHO and GEPA as shown in Table 1 below. Acidity assessment of water collected from streams within the Asanti Akim North District was 6.493. Analysis of results from these two districts (i.e. Asanti Akim North and Amansie West District) showed that, levels of arsenic and other heavy metals were not significantly different from each other (Table 2). Analysis of results also showed that, mean arsenic concentration in water collected from streams in BU endemic communities (0.0686 mgL^{-1}) of Asanti Akim North District was less compared to the non endemic counter parts (0.240mgL^{-1}). These were however not statistically significant (p¡0.05) as shown in Table 2 below but exceeded both the WHO and GEPA recommendations for arsenic in drinking water. Mean Arsenic levels recorded in sediments collected from sampled streams in BU endemic communities of the Asanti Akim North District were however greater (0.282mgL^{-1})

Table 1. : The Concentration of Arsenic (water and soil) with some Physicochemical Parameters in the Asante Akim North Municipal of Ghana

Parameters	Mean	SD	WHO/ GEPA
Arsenic (streams) mgL^{-1}	0.1545	0.1743	0.01
Arsenic (soils) mgKg^{-1}	0.1643	0.1895	-
Conductivity (uSL^{-1})	176.4	63.340	1000
Total Dissolved solids(mgL^{-1})	92.46	29.180	500
pH	6.493	0.5717	6.5-8.5

SD-Standard Deviation, WHO-World Health Organization, GEPA-Ghana Environmental Protection Agency. Endemic communities-Dukusen, Srebuoso and Ananekrom whiles non-endemic communitie-Pataban, Supinisu and Agogo .

compared to the non-endemic control (0.0705 mgL^{-1}) (Table 2). Mean TDS of water collected from communities within the district were also compared based on endemicity. Analysis of results from these physicochemical parameters also showed that, there was no significant difference as shown in Table 2. This was also seen with$_p$H (6.787) A comparative analysis of arsenic was carried out based on samples collected from a particular district (Amansie West and Asanti Akim Districts). Analysis of results from the study showed that, Amansie West recorded a mean arsenic level (0.1772 $\pm$ 0.07323mgL^{-1}) in streams and this was greater than that of Ashanti Akim North District (0.1545 $\pm$ 0.0503mgL^{-1}). The difference however was not significant (p¡0.05). Similarly, soils in the Amansie West District had mean arsenic levels (0.4517 $\pm$ 0.1078mgL^{-1}) that also exceeded their Ashanti Akim counter parts (0.1643 $\pm$ 0.0547mgKg^{-1}) as shown in Table1. This difference was however, statistically significant (p= 0.0266) with levels exceeding WHO and GEPA recommendations.

With respect to the other heavy metals, cadmium levels were stratified in water and soil based on the district and analysis of the results showed that, Amansie West District had mean cadmium levels in their streams (0.0568 $\pm$ 0.0045 mgL^{-1}) which was less than that of Asanti Akim North (0.0600 $\pm$ 0.0074 mgL^{-1}). This was not significant (p= 0.7185) as shown in Table 3. Mean cadmium levels in soils sampled in Amansie West (0.04725 $\pm$ 0.0042mgKg^{-1}) was not only greater than that of Ashanti Akim North (0.0363 $\pm$ 0.0028mgKg^{-1}) but statistically significant (p=0.0028) as shown in Table 3 below.

Mean lead levels stratification based on districts showed that, Ashanti Akim North District had levels for both streams (0.5545 $\pm$ 0.0574mgL^{-1}) and soil (0.9211 $\pm$ 0.3164mKg^{-1}) greater than that of Amansie West District (0.4608 $\pm$ 0.1527mgL^{-1}) and (0.7509 $\pm$ 0.0645mgKg^{-1}) respectively (p¿0.05). Total dissolved solids and conductivity levels as well as acidity of streams monitored for the two districts during the study were also greater for Ashanti Akim North compared to Amansie West (Table 3).

Table 2. Stratification of Arsenic and some Physicochemical Parameters in the Asante Akim North Municipal based on Endemicity

Parameter	BU endemic communities Mean ± SD	Non-endemic communities Mean ± SD
Arsenic (mgL^{-1}) Stream	0.0686 ± 0.0434	0.240 ± 0.0794^{ns}
Arsenic($mgKg^{-1}$) Soil	0.282 ± 0.09367	0.0705 ± 0.0296^{ns}
Total Dissolved Solid (mgL^{-1})	93.35 ± 30.1600	91.570 ± 31.01^{ns}
Conductivity (uS/cm)	185.4 ± 58.1000	167.40 ± 72.49^{ns}
pH of Stream water	6.787 ± 0.0580	6.2000 ± 0.4157^{ns}

SD-Standard Deviation, (N=36). Levels of significance were determined using Students unpaired t-test. ns implies $P > 0.05$; Statistically significant implies: † implies $P \leq 0.05$; ††† implies $P \leq 0.001$. Endemic communities-Dukusen, Srebuoso and Ananekrom whiles non-endemic communitie-Pataban, Supinisu and Agogo

Table 3. Showing Mean Arsenic, Cadmium, Lead and other Physicochemical Parameters in 2 Buruli Ulcer Endemic Districts in the Ashanti Region Ghana.

Parameter (Water)	Mean (SD) Amansie West	Mean (SD) Ashanti Akim North	WHO
As water (mgL^{-1})	0.1772 ±0.07323	0.1545 ± 0.0503^{ns}	0.01
As soil ($mgKg^{-1}$)	0.4517 ± 0.1078	0.1643 ± 0.0547†	-
Cd water (mgL^{-1})	0.0568 ± 0.0045	0.0600 ± 0.0074^{ns}	0.01
Cd soil ($mgKg^{-1}$)	0.04725 ± 0.0042	0.0363 ± 0.0028†	-
Pb water (mgL^{-1})	0.4608 ± 0.1527	0.5545 ± 0.0574^{ns}	0.01
Pb soil ($mgKg^{-1}$)	0.7509 ± 0.0645	0.9211 ± 0.3164^{ns}	-
Conductivity (mgL^{-1})	152.1 ± 17.48	176.4 ± 18.280^{ns}	1000
TDS (mgL^{-1})	76.44 ± 9.157	92.46 ± 8.4230^{ns}	500
pH	6.403 ± 0.0839	6.493 ± 0.1650^{ns}	6.5-8.5

SD-Standard Deviation, (n=12). Levels of Significance were determined using Students Unpaired t-test. ns implies P ¿ 0.05; (†) implies $P \leq 0.05$, (††) implies $p \leq 0.005$. Words in Parenthesis, next to the Communities refers the names of Streams where Water and Soil samples were taken, Figures in Parenthesis refers to the Standard Deviation, p-value ¡ 0.05 is considered statistically significant at 95% confidence Interval., Asanti Akim North District: Endemic communities-Dukusen, Srebuoso and Ananekrom whiles non-endemic communitie-Pataban,Supinisu & Agogo. Amansie West District: Endemic communities- Yawkasakrom, Bonsaaso and Tontokrom whiles non endemic communities-Yawhemekrom, Manso Akropong & Manso Mim

3. Discussion

This is the first time a pilot study is been carried out in 2 BU endemic districts in the Ashanti Region, Ghana to compare their levels of arsenic and other heavy metals in relation to MU infections. It has been reported earlier that arsenic in the environment could play a catalytic role in Buruli Ulcer development in Ghana [16]. The earlier study was triggered by a research conducted in the Amansie West District of Ghana using spatial modelling to establish the correlation between arsenic enriched domains as well as farmlands and BU infections [14]. Results from the study lead to the prediction that, the Amansie West District could be polluted with arsenic. In a follow up study, Gyasi *et al.* (2012b) monitored arsenic in water and soil collected from streams in BU endemic communities of the same district (Amansie West) over a period of 24 months. Analysis of the results confirmed, the district was polluted with arsenic.

It was further proven, levels of arsenic in drinking water, synonymous to concentrations recorded in BU endemic communities of the Amansie West District made ICR (Imprinting Control Region) mice susceptible to MU infections [16]. The next research question which needed to be unraveled was that, “Is arsenic present in other BU endemic communities outside the Amansie west District”? And “At what concentrations do they occur”? In addition, the question of whether Arsenic was the only heavy metal which could be involved in BU pathogenesis also needed to be answered. To provide an answer to this dilemma, we sought to undertake a comparative study of arsenic and other heavy metals in 2 BU endemic communities in the Ashanti Region of Ghana.

The high mean arsenic concentration in streams of the study communities in Asanti Akim North District, which exceeded the WHO and the GEPA recommendations for arsenic in drinking water, was highly anticipated. In-depth interview conducted with the Disease Control Officer (DCO-Asanti Akim North) in charge of Buruli Ulcer during data collection indicated, the emergence of the disease coincided with major disturbances of the soil within the affected communities during an extensive highway road construction some 15 years ago *(Abass pers. Comm. 2012)* . Research has shown that, Buruli Ulcer has occurred after the environment had undergone massive disturbance e.g. in the contraction of dams, farming and creation of recreation facility [17]. It has been proven that, arsenic in the form of pyrites and arsenopyrites are exposed to air and oxidized with major soil disturbance. With the onset of the rains, they are washed into farmlands and other drainage channels [18].

Our study showed that, there was lack of significant difference between arsenic concentrations detected in BU endemic and their non-endemic control communities within the Asanti Akim North District. This could be understood as, it has been hypothesized earlier [15], arsenic alone in the environment could not possibly cause BU. In a non endemic community, the presence of arsenic may not have a significant impact with respect to BU. However, in the presence arsenic in drinking water at a given critical concentrations in BU endemic communities, arsenic in drinking water could act as a catalyst for the on-set of infection. This was consistent with a study conducted by

The non significant difference between arsenic levels detected in water collected from streams in the Asanti Akim North and that of Amansie West District also met our expectation. These concentrations however, were high enough to exceed the limits set by WHO and GEPA. This was in confirmation of the fact that, arsenic in water and soil could play a catalytic role in BU infection [15]. Duker et al., (2004) reported previously that, arsenic enriched domain in the Amansie West District of Ghana was a possible covariant to *Mycobacterium ulcerans* infection. Work carried out by earlier researchers has shown that, arsenic occurs naturally and is widely distributed in the environment [3]. Its association with some non-weathering-resistant mineral deposits (e.g., sulphide minerals) has contributed to its release in large amounts into the environment especially when present in large quantities in the soil after they are heavily disturbed [2].

In his study of host susceptibility factors of BU, Stienstra indicated *Mycobacterium ulcerans* alone could not induce systemic immunosuppression. He further stressed other factors could possibly play a significant role which could either be genetic or environmental, establishing the possible role of arsenic involvement in BU pathogenesis [20]. The significantly lower arsenic concentration in soil in the Asanti Akim North District compared to that of the Amansie West could be attributed to the absence of artisanal gold mining activities in the district. Research has shown that, mining activities cause arsenic to be released in high concentrations from oxidized sulphide minerals [21]. This may have resulted in relatively higher concentrations of arsenic in surface water soil, vegetation and groundwater in the Amansie West District than Asanti Akim North [22].

High levels of cadmium and lead recorded for both streams and soils around streams in the two BU endemic communities (i.e. Amansie West and Asanti Akim North District) was quite worrying. The implication of these heavy metals (Cd and Pb) to MU infections in the Ashanti Region of Ghana has not been fully explored and this warrants urgent research. Little research has been done on dermal absorption of cadmium and Lead. In 1991, Del Razo et al. experimented on the resorption from cadmium-contaminated soil and water solutions by human cadaver skin in a diffusion cell-model. They could demonstrate a penetration of 8.8 % (soil) and 12.7% (water) of the applied cadmium dose into the skin; while the plasma uptake from soil was 0.01% and 0.07% from water [23]. The skin showed hyperkeratosis and acanthosis with occasional ulcerative change, and this resulted in an increase of the mitotic index of the skin cells. In addition, cadmium concentration in blood, liver and kidney increased, thus indicating percutaneous absorption [23]. But as to whether or not cadmium has a role to play in the pathogenesis of BU is yet to be fully investigated.

4. Conclusion

Result from this pilot study has shown that the Asanti Akim North District is also polluted with arsenic just as the Amansie West, all in the Ashanti Region of Ghana. This assertion lay credence to the fact that the involvement of arsenic in the environmental as a catalyst for BU infections cannot be overlooked. The present study could support the involvement of arsenic in Buruli ulcer infection pathogenesis. It is therefore recommended that a longitudinal study involving arsenic analysis be carried within the Asanti Akim North District to unravel the extent of the pollution in the Region.

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